

the AFIP LETTER

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AFIP Scientists Explore Gulf War Illness Theories

(Photo below) Division of Molecular Pathobiology: (from left) Michael Hayes, microbiology technician; Susan Ditty, molecular biology technician; Jose Rodriguez, laboratory technician; Shyh-Ching Lo, MD, PhD, chief, Division of Molecular Pathobiology; Bing Jie Li, molecular biology technician; and Mike Connolly, mycoplasma research assistant.

(Photo far right) Biochemistry Division: (from left) John Davis, BA; Sonia Neves, MD; William Fishbein, MD, PhD; Natasha Merezhinskaya, PhD; and John Foellmer, BS.

The Armed Forces Institute of Pathology is joined with the Walter Reed Army Medical Center (WRAMC) Gulf War Health Center, which is supported by the Henry M. Jackson Foundation for the Advancement of Military Medicine, in exploring the etiologies and characteristics of the diverse medical conditions that have collectively been called the Persian Gulf Illness (PGI). According to AFIP Director Michael J. Dickerson, Col, USAF, MC, the AFIP continues to play a significant role in assisting the Department of Defense and Department of Veterans Affairs as well as the affected servicemembers in understanding and analyzing this illness through a comprehensive multidisciplinary, multifactorial laboratory investigation of the blood and tissue specimens obtained from servicemembers who served in the Gulf. Two AFIP departments have been instrumental in the AFIP's efforts to address these critical issues of Gulf War veterans who are presenting with unexplained signs and symptoms.

According to Florabel G. Mullick,

MD, SES, AFIP Associate Director and Director, Center for Advanced Pathology, "Dr. Shyh-Ching Lo in Molecular Pathobiology is looking for evidence of mycoplasma infections, and Dr. William Fishbein in the Environmental Pathology Biochemistry Division is studying the potential impact of environmental agents on chromosomal stability."

Dr. Lo's laboratory has been working on various mycoplasmal diseases for more than 10 years. In the late 1980s, he and his colleagues first reported the finding that many patients with AIDS were infected by unusual species of mycoplasma. The commonly dubbed *Mycoplasma incognitus* was subsequently characterized as a strain of *Mycoplasma fermentans*. The significance of infections by these mycoplasmas is still not clear. Dr. Luc Montagnier of The Pasteur Institute, Paris, later confirmed the finding and proposed that mycoplasmas may be an important co-factor of AIDS disease. Subsequently, a previously unknown human mycoplasma was also identified, characterized, and named *Mycoplasma penetrans* by Dr. Lo and his associates. *M penetrans* mainly

Gulf War, continued on page 10



Photos: Chris Kelly

DIRECTOR'S MESSAGE



Supporting the Department of Defense through Strategic Planning

The Armed Forces Institute of Pathology continues to play a vital role in supporting the Department of Defense (DoD) through a variety of initiatives based on pathology and laboratory medicine applications in support of military operations, readiness, and healthcare. While every AFIP department supports the services in their consultation, education, and research efforts, there are several departments and programs that have been identified as the AFIP's readiness pillars: (1) the Armed Forces Medical Examiner's System, (2) the Department of Infectious and Parasitic Diseases Pathology, (3) the Department of Environmental and Toxicologic Pathology, (4) the Department of Veterinary Pathology, (5) the Telemedicine Program, and (6) the developing DoD Genetic Program. These areas of expertise and resources are by definition multidisciplinary and multifactorial. The Institute's participation in the investigation of the Gulf War Illness (PGI) is one such effort.

The application of such resources to relevant military issues is a fundamental aspect of the AFIP's comprehensive strategic planning program, which is in its fifteenth month of development. The AFIP has a distinctive ability to bring triservice resources together to address these issues and other DoD responsibilities to the health and well-being of our military personnel.

From a research perspective, the AFIP will continue to provide its rich and varied resources in environmental pathology, toxicology, occupational diseases, infectious diseases, forensic investigations of current and historic materials, and clinical and laboratory genetics, as well as diagnostic molecular biology and pathology. Two major areas of focus are:

- Responding to operational fatal mishaps involving military aircraft, ships,

Senate Staffers Visit AFIP's Telepathology Program



Paul A. Fontelo, LTC, MC, USA (rear), Telepathology Program Manager, and Leslie H. Sobin, MD, SES, chief, Division of Gastrointestinal Pathology, review a telepathology image.

Staffers from five U.S. Senate offices paid an October 21, 1996, visit to the Armed Forces Institute of Pathology to learn more about its Telepathology Program. The ASCP-sponsored visit provided the staffers who represented Senators Harkin, Grassley, Burns, Jeffords, and Dorgan, with additional information on the benefits of telepathology for remote locations.

AFIP Director Michael J. Dickerson, Col, USAF, MC, welcomed the group and provided an update on Institute initiatives in the telepathology arena. "The greatest value of telepathology in present-day medical care is the speed in obtaining expert diagnostic consultation over remote distances, and we've been very successful with this at the AFIP," he said.

Paul A. Fontelo, LTC, MC, USA, Telepathology Program director, encouraged the staffers to consider AFIP for future collaborations with hospitals in their states. "Their primary concern," he noted, "was to learn more about telepathology resources for rural hospitals. We provided

a thorough briefing on how it's done, the types of hardware and software needed, and the success we've had receiving images from remote sites in the United States and around the world." AFIP accepts telepathology cases through a modem connection, or through generic file transfer protocol (ftp) on the Internet. Because commercially available telepathology systems require both parties to use the same hardware and software, AFIP receives most of its cases through the Internet. "We're able to FAX the final diagnosis within 1 to 4 hours after images are received," Fontelo told the visitors, adding that "real-time telepathology is somewhat limited, especially due to time-zone and hardware differences between the contributor and the Institute."

The staffers got a close-up look at telepathology images, as Fontelo described the value of the program. "With so many changes going on in health care, telepathology will be an indispensable diagnostic modality for the future," he said.

and land vehicles with the Armed Forces Medical Examiner's System and working with the respective service mishap investigation board and service safety centers to identify the mishap causes and the procedures or equipment that will minimize injury and/or prevent similar mishaps.

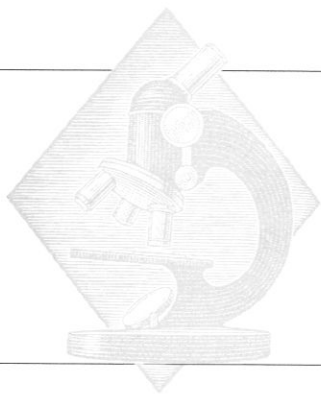
- Providing a principal source of advanced medical laboratory support for DoD deployed forces through improved surveillance, reference laboratories, and telemedicine including telepathology, teleradiology and teleforensics. The Institute in collaboration with other agencies has a unique opportunity to identify and study biomarkers of biological

and chemical toxicity.

Our cover story outlines the Institute's efforts to provide insight into the causes and nature of the Persian Gulf Illness and its perceived relevance in this multiagency, multidisciplinary effort. The described effort addresses the multiple capabilities of the AFIP and its commitment to the health and well-being of its constituencies.

Michael J. Dickerson
 Michael J. Dickerson
 Col, USAF, MC
 The Director

Photo: Chris Kelly



Interdisciplinary Course on Breast Disease, April 25-26 1997, Leavey Conference Center, Georgetown University

AFIP will present a short interdisciplinary course on breast disease from April 25-26, 1997, at the Leavey Conference Center, Georgetown University, Washington, D.C. "Breast cancer is the second leading cause of death in American women and is an extremely current, and for some aspects, controversial topic," says course director Sally-Beth Buckner, SCT(ASCP), IAC, Department of Cellular Pathology. The recent NCI Breast Fine Needle Aspiration Conference and the NIH Consensus Development Conference on Breast Cancer Screening in Women Ages 40-49 attest to the need and interest in this area. Because it is imperative that the most cost-effective and efficacious methods of detection and procurement are used and the most beneficial means of treatment are rendered, controversial topics such as procurement and treatment must be addressed. This conference is designed to disseminate information and foster

communication among practitioners of different specialties dealing with breast disease.

Formal lecture sessions presented by a panel of experts in their respective fields will cover imaging, fine needle aspirations, stereotactic needle biopsies, surgical pathology, molecular pathology, surgical treatment and management, limitations of aspiration diagnoses, the role of molecular pathology, and patient treatment and management. Practical application of this multidisciplinary approach will be presented with the discussion of actual cases by speakers during a panel session.

It is hoped that course participants will more fully understand radiologic and pathologic correlation for early detection of breast cancer, cytologic diagnoses and limitations of fine needle aspirations, uses and limitations of molecular biologic techniques, surgical treatment and management of breast disease, when chemotherapy

is the treatment of choice, and medicolegal considerations of breast disease.

Speakers are renowned in their fields and are associated with the Armed Forces Institute of Pathology, the National Naval Medical Center, Columbia Hospital for Women, the George Washington University Medical Center, the University of Maryland, and the East Carolina University School of Medicine.

The Leavey Center is a gracious, full-service conference center located on the beautiful historic campus of Georgetown University in Washington, D.C. Rooms are reserved at a special rate of \$108.41 plus tax until April 4, 1997. Tuition for the conference is \$295. Questions concerning the course may be addressed to SSgt Lawrence Austin, Department of Education Services, AFIP, 1-800-577-3749, or commercial (202) 782-5021/9280.

1996 Dermatopathology Workshop Highlights

AFIP's Department of Dermatopathology conducted "The Dermatopathology Workshop" on November 8-9, 1996, at the Ritz-Carlton Hotel in Arlington, Va. "We're pleased to report that the course was filled to capacity and even had a waiting list," says course director and department chair George P. Lupton, MD. Joining Dr. Lupton from AFIP's Department of Dermatopathology were Maria-Magdalena Tomaszewski, COL, MC, USA; Luke S. Chong, COL, MC, USA; John C. Moad, MAJ, MC, USA; and Sylvana M. Tuur-Saunders, MD. Henry G. Skelton, MD, recently retired from the U.S. Navy, also participated.

"One hundred dermatopathology cases were presented for study each day, and each case was accompanied by a multiple choice question," Dr. Lupton points out. For selected cases, the diagnosis with discussion of the case was

printed on the reverse side of the question sheet. Other cases were presented as "unknowns," with only the multiple choice question. The "unknown" cases were discussed formally by the faculty in the afternoon sessions.

"The response of the participants to the Dermatopathology Workshop was

extremely positive and is indicative of the strong preference of many pathologists to attend meetings where they can examine microscopic slides as a hands-on experience," says Dr. Lupton.

The Dermatopathology Workshop will be offered again on November 7-8, 1997, at the Ritz-Carlton Hotel, Arlington, Va. Mark your calendars now and make *early* plans to attend this challenging and exciting educational event.

Faculty for the Dermatopathology Workshop (L to R): Drs. Tuur-Saunders, Tomaszewski, Moad, Lupton, Skelton, and Chung.



PROFILE



RICHARD K. HARRIS, COL, USAF, BSC, retired from active duty in December 1996, as chair, Department of Veterinary Pathology, to accept a position as associate director, Product Safety Assessment at G.D. Searle, in Skokie, Illinois. A native of Texas, Colonel Harris was raised in an Air Force family and graduated from Airline High School in Shreveport, Louisiana. He earned a BS degree and a DVM in 5 years and graduated from Texas A&M University in 1976.

Col Harris attended veterinary school under the Health Professions Scholarship Program and entered active duty as a captain in the U.S. Air Force Veterinary Corps. His first permanent duty station was

Richard K. Harris, Col, USAF, BSC, retires as Chair, Department of Veterinary Pathology

Clark Air Base, Philippines, from 1976 to 1978, where he oversaw veterinary care for over 140 military working dogs, managed the largest veterinary clinic in DoD, and managed an extensive food safety program. His next assignment took him back to Colorado at Lowry Air Force Base in Denver, where he oversaw the care of military working dogs, animal medicine for privately owned animals, and food safety and quality assurance programs.

Col Harris then completed the Veterinary Pathology Residency Program at the AFIP from 1980 to 1983. He was then assigned at Brooks Air Force Base, Texas, from 1983 to 1987 as chief, Anatomic Pathology. This position included services for a large biomedical research and education program and support for a primate colony.

In 1987, Col Harris returned to the AFIP as chief, Division of Veterinary Pathology. His responsibilities included the operation of the DoD's residency program in veterinary pathology, a large consultation service, and an international educa-

tional center. Since 1992, he has served as chair, Department of Veterinary Pathology, managing the department's consultation, education, and research missions; coordinating the Institute's laboratory animal program; and serving as registrar of three pathology registries. He served as consultant to the Air Force Surgeon General for Veterinary Pathology; as associate corps chief for Veterinary Science, USAF Biomedical Sciences Corps; and as president, American Registry of Pathology Registrar's Forum.

Col Harris is a diplomate of the American College of Veterinary Pathologists (ACVP) and a member of many professional organizations. He has been a member of the ACVP Examination Committee since 1993. He is the author or coauthor of numerous scientific publications.

He and his wife Bonnie have three sons, Bryan, Richard, and William.



Photo: Chris Kelly

Lenore Barbian, PhD, Collections Manager, National Museum of Health and Medicine, provides a behind-the-scenes look at skulls, plastinated specimens, and Civil War specimens to MG Leslie Burger, Commander, Walter Reed Army Medical Center. MG Burger toured AFIP and the Museum with Col Michael J. Dickerson, AFIP Director (R), on December 26, 1996.

AFIP staff prominent at XXI International Congress, IAP (International Academy of Pathology)

The XXI International Congress of the International Academy of Pathology (IAP) was held in the beautiful city of Budapest, Hungary, October 20-25, 1996. Over 1,700 pathologists from 67 countries attended, with the largest number of participants from Hungary, the United States, and Japan. **Florabel G. Mullick, MD, SES**, AFIP Associate Director and Director, Center for Advanced Pathology, headed an AFIP staff contingent that actively supported the scientific program. She and **Kamal G. Ishak, MD, PhD**, chair, Department of Hepatic and Gastrointestinal Pathology, conducted and participated in a short course on the "Pathology of Drug-Induced Liver Injury." **Dr. Mullick** also chaired an update lecture on cytopathology given by D. L. Rosenthal, MD, of Baltimore, Md. **Cesar A. Moran, Maj, USAF, MC**, associate chair, Department of Pulmonary and Mediastinal Pathology, organized and cochaired a long course on "Neoplastic Thoracic Pathology," at which both he and **Michael Koss, MD**, cochair, Department of Pulmonary and Mediastinal Pathology, presented lectures. **Renu Virmani, MD**, chair, and **Allen Burke LtCol, USAF, MC**, associate chair, Department of Cardiovascular Pathology, organized and participated in a symposium entitled "Current Concepts in Cardiovascular Pathology." **Fattaneh A. Tavassoli, MD**, chair, Department of Gynecologic and Breast Pathology, and **Leslie H. Sobin, MD, SES**, chief, Division of Gastrointestinal Pathology, Department of Hepatic and Gastrointestinal Pathology, participated in another symposium on the "Pathology of Incipient Neoplasia," while **Timothy O'Leary, MD, PhD**, chair, Department of Cellular Pathology, gave a talk on flow cytometry at a symposium entitled "Special Techniques in Clinical Cytology."

William D. Travis, MD, cochair, Department of Pulmonary and Mediastinal Pathology, **Kamal G. Ishak, MD, PhD**, chair, Department of Hepatic and

Gastrointestinal Pathology, **George P. Lupton, MD**, chair, Department of Dermatopathology, and **Isabell A. Sesterhenn, MD**, senior pathologist, Department of Genitourinary Pathology, presented cases at slide seminars on "Interstitial Lung Diseases," "Liver Pathology," "Dermatopathology," and "Uropathology," respectively. **Dr. Travis** chaired his slide seminar, as did **F. K. Mostoffi, MD**, chair, Department of Genitourinary Pathology.

Free papers were presented by **Julie C. Fanburg, MD**, Department of Soft Tissue Pathology (extrarenal (soft tissue) rhabdoid tumors), **William D. Travis, MD**, cochair, Department of Pulmonary and Mediastinal Pathology (survival in idiopathic pulmonary fibrosis), **F. K. Mostoffi, MD**, chair, Department of Genitourinary Pathology (frequency and location of benign prostatic glands in the surgical margin of 147 radical retropubic prostatectomies for carcinoma), **Isabell A. Sesterhenn, MD**, senior pathologist, Department of Genitourinary Pathology (interphase cytogenetics in transitional cell carcinoma in situ and associated concomitant bladder tumors), and **Lester D. R. Thompson, LCDR, MC, USNR**, staff pathologist, Department of Otolaryngic and Endocrine Pathology (branchial cleft cyst carcinoma). Abstracts by members of the staff were published in the proceedings (six by **Isabell A. Sesterhenn, MD**, and one each by **Julie C. Fanburg, MD**, **Lester D. R. Thompson, LCDR, MC, USNR**, **F. K. Mostoffi, MD**, and **William D. Travis, MD**). Three poster presentations were given by **Dr. Sesterhenn**. At the ARP/AFIP booth, manned by **Mr. Jonathan Johnstone**, marketing director, American Registry of Pathology, 109 copies of the AFIP fascicles (and other special publications) and 27 CD-ROMs, were sold to participants.

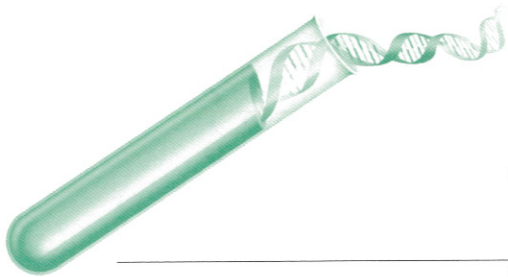
The overall caliber of the scientific



Florabel G. Mullick, MD, SES, AFIP Associate Director, and Secretary, International Academy of Pathology, presents the AFIP Medallion to Anna Kadar, MD, President of the XXI International Congress of the IAP.

presentations was excellent. The social program included an entertaining opening ceremony, a lavish buffet reception at the Hungarian National Gallery, a banquet at the Hotel Hilton, and many optional programs.

The business meeting was held on the last day, with the president, treasurer, and the secretary (Florabel G. Mullick, MD, SES) and other functionaries presenting their reports, followed by adjournment. The next International Congress of the IAP will be held in Nice, France, in October 1998.



Nuclear DNA Section Provides Medical Examiner Support

When the Office of the Armed Forces Medical Examiner (OAFME) conducts the grim task of identifying victims of aircraft accidents, training incidents, or other unusual or suspicious deaths, the Nuclear DNA Section at AFIP's Armed Forces DNA Identification Laboratory (AFDIL) is there to support them. According to Dr. Jerry Spencer, the Armed Forces Medical Examiner, the Nuclear Section provides OAFME with an additional method of positive victim identification. "We are now utilizing DNA techniques to support the traditional methods of fingerprint and dental identification in these cases, and it's really proved valuable," he says.

Under the direction of Victor W. Weedn, LTC(P), USA, chief deputy medical examiner, AFDIL is a key component of the DoD DNA Registry. The registry also includes the Armed Forces Repository of Specimen Samples for the Identification of Remains, located in nearby Gaithersburg, Md. AFDIL's service laboratory branch is headed by Mitchell M. Holland, PhD, and is comprised of two main sections. One is dedicated to nuclear DNA testing and the other to mitochondrial DNA testing. Both sections provide DNA testing services for human remains identification of military personnel.

The nuclear section is comprised of the chief DNA analyst, Demris A. Lee; two DNA analysts, Jeanne Willard and Richard Wilson; and technologist Jim Ross. "The members of the nuclear DNA section are a cohesive team of forensic scientists, and are ready to respond to any OAFME request at a moment's notice," says Dr. Holland. "I am truly proud of their accomplishments and dedication."

According to section chief Demris Lee, the Nuclear Section has been incorporated into the OAFME 'team' that deploys to accidents or incidents in the field. "Our goal is to collect specimens right away and have a DNA identification completed before the autopsies are



Reviewing a case are (L to R) Demris A. Lee, Richard Wilson, Jeanne Willard, and Jim Ross.

completed."

Lee says that by having someone on the OAFME team (a group that usually consists of forensic pathologists, forensic anthropologists, odontologists, fingerprint examiners, criminal investigators, photographers, and administrative personnel), she knows up front how many specimens to expect. "That way, as soon as samples arrive, we'll have appropriate personnel here to work through the night and make an identification," she says.

The Nuclear Section's work is important to the families of the victims as well. "They want their loved ones back immediately, but until a positive identification is made, a death certificate can't be signed," Lee says. "Utilizing DNA as an option helps ensure a timely identification."

When an accident or incident occurs, tissue specimens are sent by the responding OAFME team. To make a positive DNA identification, the Nuclear Section utilizes a tiny bloodstain reference sample that is on file in the Armed Forces Repository of Specimen Samples for the Identification of Human Remains. The

repository contains over 1.6 million bloodstain cards and oral swabbings, collected from servicemembers since 1992. The victim's card is pulled, and a nuclear DNA analysis is completed. The DNA profile obtained from the tissue specimen is compared to the DNA profile obtained from the bloodstain reference.

The section currently utilizes PolyMarker analysis (reverse dot blot) and short tandem repeat (STR) analysis. PolyMarker analysis is done first because it provides quick preliminary results. The PolyMarker results are confirmed with the STR process, because STRs are more discriminating. The identification is based on a combination of the two. For the majority of the cases processed in the past two years, results were obtained within 24 hours from receipt of the specimens. In 1996, the Nuclear Section completed DNA analysis on 40 military cases and conducted 1,200 random quality assurance cases on specimens kept in the repository.

The nuclear DNA section is trained in many areas and all members routinely perform such functions as logging-in and

Continued on page 7

PROFILE



Gael J. Lonergan, Major, USAF, MC, appointed as pediatric radiologist in the Department of Radiologic Pathology

University of the Health Sciences, she will provide radiologic anatomy and pathology instruction to first- and second-year medical students and teach diagnostic radiology to second- and fourth-year students.

Major Lonergan was most recently stationed at Wilford Hall U.S. Air Force Medical Center, where she worked as a pediatric radiologist since 1992. She served as assistant program director for the radiology residency training program and received Teacher-of-the-Year honors in 1993. She completed her diagnostic radiology residency at Wilford Hall U.S. Air Force Medical Center in 1992, and served as chief resident during her last year of residency. She was certified by the American Board of Radiology in June of 1992.

Her military career began when she joined the class of 1985 at the Uniformed Services University of the Health Sciences. She completed a general surgery internship at Wilford Hall U.S. Air Force Medical Center, followed by a 2-year tour of duty at Hellenikon Air Base, Athens, Greece, as a general medical officer.

Major Lonergan's area of special interest is pyelonephritis, and she received

1st place honors at the International Pediatric Radiology Meeting in 1996 for her work on this subject. She pioneered the use of magnetic resonance imaging for the diagnosis of pyelonephritis. Major Lonergan is also interested in the radiologic diagnosis of child abuse and serves as expert witness at numerous military legal actions. She is currently designing an Internet education, consultation, and information resource on child abuse for use by military physicians, nurses, judge advocate generals, law enforcement officers, and special investigators to improve timely and effective evaluation of suspect cases.

Major Lonergan was awarded the Meritorious Service Medal for her service to the Department of Radiology at Wilford Hall U.S. Air Force Medical Center and has been selected for promotion to Lieutenant Colonel. She is a member of the Society for Pediatric Radiology, the Radiological Society of North America, the American College of Radiology, the American Roentgen Ray Society, and the American Association for Women Radiologists.

Major Lonergan is married to Douglas Lair. They live in Olney, Maryland.

GAEL J. LONERGAN, MAJOR, USAF, MC, was appointed to the Department of Radiologic Pathology as a pediatric radiologist on August 19, 1996, and has been selected to serve as chief of Pediatric Radiology. She is stationed at the Uniformed Services University of the Health Sciences in the Department of Radiology and Nuclear Medicine and works at the AFIP through a memorandum of understanding between AFIP and USUHS. Major Lonergan will participate in all the educational and research activities of the Department of Radiologic Pathology, to include teaching in the 6-week Radiologic Pathology Course, which is attended by over 1,200 radiology residents and radiologists each year. She will provide consultation in pediatric radiology and will work in the Pediatric Pathology Education Center. At the Uniformed Services

DNA, Continued from page 6

photodocumenting evidence, in addition to completing the skilled technical analysis necessary to obtain an identification. The Nuclear Section is also prepared in the event of a mass disaster by utilizing the talents of AFDIL's larger Mitochondrial Section (a staff of 22 whose primary mission is to identify older, degraded remains of servicemembers killed in Southeast Asia). "We have teams of analysts, technicians, and administrative personnel prepared to handle a mass-fatality incident," Lee says.

"We know how important it is to provide family members with a timely,

accurate identification of their loved one, and that's why we routinely cross-train our personnel."

In addition to performing OAFME cases, the Nuclear Section reviews pathology slides and paraffin blocks for potential sample switches in the diagnostic process; participates in the Federal Bureau of Investigation (FBI) on a new STR Standardization Project for the forensic community; and serves as a resource laboratory for national and international cases in need of expert STR analysis.



HISTOTECHNOLOGY NOTES**WENGER-ANGRITT PROCEDURE**
for Some Argyrophilic Microorganisms

PURPOSE: This modification of Manuel's reticulum demonstrates and helps in the screening of some argyrophilic microorganisms. It is a synthesis, with modifications, of several silver impregnation methods, notably Gomori's original method and Manuel's reticulum procedure as found in the AFIP technical manual. It uses the permanganate-metabisulfite sequence to clear the background, followed by uranium nitrate sensitization and a modified Fontana's ammoniacal silver for impregnation. The wash following the silver impregnation contains the reducing agent (formalin) in order to minimize the loss of silver. The treatments with gold chloride and sodium thiosulfate (hypo) are omitted to optimize bacterial staining.

FIXATION: 10% buffered neutral formalin.

MICROTOMY: Paraffin sections, cut at 6 micrometers.

CONTROLS: Material containing the target bacteria.

SOLUTIONS:**0.25% Potassium Permanganate**

Potassium permanganate crystals 1.0 gm
Deionized water 400.0 ml

1% Sodium Meta Bisulfite

Sodium metabisulfite crystals 1.0 gm
Deionized water 100.0 ml

1% Uranium Nitrate

Uranium nitrate crystals 1.0 gm
Sterile Water for Irrigation, USP 100.0 ml

Reducer/Rinse

Concentrated formalin (37-40%) 50.0 ml
Hot (60°C) tap water 1000.0 ml

10% Silver Nitrate

Silver nitrate crystals 10.0 gm
Deionized water 100.0 ml

Stock Fontana Ammoniacal Silver

Add 10% silver nitrate to 50 ml of concentrated ammonium hydroxide in an Erlenmeyer flask on a magnetic stirrer until the solution is opalescent (tan and slightly cloudy). Solution should have only a weak ammonia odor. Refrigerate overnight before using.

Working Fontana Solution

Stock fontana solution 45.0 ml
Hot tap water 5.0 ml

Stir and allow to sit *on top* of 60°C until the solution is a pinkish tan. It is evidently the chlorine in the tap water that creates the effect because 5 ml of dilute sodium hypochlorite (a 5.25% solution diluted 1:1000 with distilled water) also works.

STAINING PROCEDURE:

1. Deparaffinize and hydrate to deionized water. Rinse in two more changes of deionized water.
2. Oxidize in 0.25% potassium permanganate for 1 minute.
3. Reduce and clear oxidation by-products in 1% sodium metabisulfite for 1 minute.
4. Rinse 4 times in deionized water and once in sterile water for irrigation, USP.
5. Sensitize in 1% uranium nitrate 8 minutes.
6. Rinse quickly in 3 changes of deionized water.
7. Impregnate in the working Fontina solution for 1 minute, pour off, and allow slides to drain for 10 to 15 seconds.
8. Dip slides individually in a 500-ml container of freshly made, 60°C, reducer/rinse with *vigorous up and down motion* for about 5 seconds. Be sure to immerse slides *completely*. Collect them in a staining jar containing reducer/rinse. Do not run more than 15 slides in each 500 ml of the reducer/rinse.
9. Slides should be left in the reducer at least 1 minute. Do not process more than 15 slides at a time. **CHECK MICROSCOPICALLY!** Reticulum and bacteria should be black at this point.
10. Rinse in warm tap water.
11. Dehydrate, clear, and mount in resinous mounting media.

RESULTS:

Argyrophilic organisms, e.g. Cat Scratch Bacilli brown to black
Reticulum black
Nuclei dark brown
Background yellow to tan

REFERENCES:

1. Luna LG, ed. *Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology*. 3rd ed. New York, NY: McGraw-Hill Book Company; 1968:89-90.
2. The Manuel's reticulum procedure was developed by Mr. Benedicto Manuel, Jr., Histopathology Laboratory, AFIP, Washington, DC 20306.

REPOSITORY AND RESEARCH SERVICES

Sending cases by express mail?

Follow these instructions

AFIP receives between 100 and 200 express delivery packages in all shapes and sizes on a daily basis. Most are delivered directly to the Receiving and Accessions Division by the various courier services. All cases being submitted for consultation should be addressed directly to the Receiving and Accessions Division, Room G071, 6825 16th Street, N.W., Washington, D.C. 20306-6000. They should **not** be addressed to individual pathologists or departments. If contributors want to address comments to an individual pathologist or department, they can do so on the AFIP contributor form or on a separate letter within the package. Addressing cases to a specific pathologist or department will result in delays in processing, since most express mail is delivered first to the Receiving and Accessions Division, sorted, and then all packages addressed to individuals are delivered to the addressee. If the individually addressed package contains case material, it will have to be sent back to the Receiving and Accessions Division for accessioning. If a package does need to be addressed to an individual or department, the inclusion of a room number in the address will facilitate timely delivery.

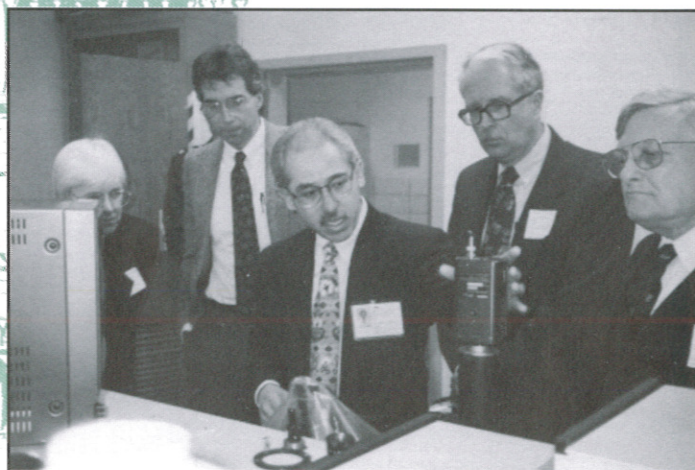
Progress is being made on the addition to our repository warehouse. June 1997 is still the targeted completion date, and more information will be forthcoming as the month draws near. Additionally, we recently installed new shelving to house a majority of material received from closed military facilities. Use of this shelving allowed us to remove the material from the shipping pallets and organize it by facility, material type, and year of origin. This has significantly improved our response time in fulfilling retrieval requests.

A new relationship between AFIP and the *Société Française de Radiologie*

Members of the Department of Radiologic Pathology at the Armed Forces Institute of Pathology participated for the first time in the Refresher Course Program of the *Journées Françaises de Radiologie* at the annual meeting of the French Radiologic Society, *Société Française de Radiologie*, which took place in Paris, November 4-8, 1996. Melissa L. Rosado de Christenson, Lt Col, USAF, MC, chair and registrar of the Department of Radiologic Pathology; James G. Smirniotopoulos, MD, chair of the Department of Radiology and Nuclear Medicine, USUHS; and Pablo R. Ros, MD, Berlex visiting professor at the AFIP delivered nine lectures in radiologic-pathologic correlation of thoracic, central nervous system, and gastrointestinal diseases at the congress, which was attended by over 13,000 radiologists and radiologic technologists from France, neighboring European countries, and Canada.

AFIP's participation in the *Journées*

Françaises de Radiologie marks the beginning of a new collaboration between the Department of Radiologic Pathology and the *Société Française de Radiologie*. AFIP faculty will continue to participate in the *Journées Françaises de Radiologie* on an annual basis. The *Société Française de Radiologie*, in collaboration with *Nycomed, Inc.*, will award a series of scholarships to allow French radiology residents to attend the 6-week Radiologic Pathology Course, thus supporting the international educational programs of the department and providing the opportunity for young French radiologists to study the principles of radiologic pathologic correlation taught to over 1,100 radiology residents from the United States and other countries. The first three scholarship recipient residents will be selected in mid-January and will attend the April 1997 Radiologic Pathology Course in the Elias G. Theros Auditorium of the Radiologic Pathology Education Center.



Jose A. Centeno, PhD, (center) research chemist, Department of Environmental and Toxicologic Pathology, demonstrates the use of laser Raman microspectroscopy for the study of tissue and medical devices to visitors from the Food & Drug Administration (FDA) in January. FDA and AFIP are considering potential collaborative programs on the study of biomedical devices.

Gulf War, Continued from page 1

infects male homosexuals in the United States. Dr. Lo's group also found rare cases of previously healthy non-AIDS patients who also became infected by *M fermentans* and became seriously ill. Independent researchers who studied "Gulf War Illness" have recently claimed that 50% to 60% of the veterans with the illness are found to be infected by *M fermentans* (incognitus strain).

Dr. Lo and his colleagues are looking for mycoplasma infections by studying blood, oral, and rectal swabs taken from symptomatic Gulf War veterans by culture and by polymerase chain reaction. "In 90 cases we've looked at so far, we haven't isolated or identified *Mycoplasma fermentans*," he says. Lo points out, "Although mycoplasma isn't a bad candidate to associate with the symptoms that the Gulf War veterans reportedly experience, so far we have nothing to show

that this group of patients has a higher incidence of infection by *Mycoplasma fermentans* as compared to the general population."

A larger serological study of over 7,000 veterans compares those who are healthy to those who have symptoms. "We're looking at serum drawn before and after the Gulf War to identify any seroconversion for a particular species of mycoplasma. It's specifically designed to see if these veterans have serological evidence of increased incidence of infection by *Mycoplasma fermentans*." That study isn't expected to be completed in October 1997.

In the Department of Environmental Pathology, Dr. William Fishbein and his staff in the Biochemistry Division are looking at potential chromosomal changes to determine if Gulf War veterans were exposed to environmental agents. In past studies, Fishbein's group has utilized a nested PCR assay to determine if one cell

out of many thousands is different. "We are using this assay to look at a particular recombined T-cell receptor that can only be formed by a chromosome translocation and occurs in normal people at a rate of less than one in 10,000 cells," he says. The receptor is not harmful, but can be used as a biomarker for certain types of translocations. "People with certain rare genetic diseases, who are known to have chromosomal instability and a high frequency of lymph gland cancers as they mature, have this receptor at a frequency 70 times higher than normal, healthy people." Another study of U.S. farmers who sprayed crops with insecticides in the Midwest found that they had an incidence of the anomalous T-cell receptor that was 20 times higher than normal. "The argument here, of course, is that environmental agents could produce the effect as a short-term marker."

As a result, the Department of Veterans Affairs recently asked Fishbein's group to look at eight Gulf War veterans who had symptoms. Intriguingly, AFIP found a frequency of hybrid chromosome receptors that was several times higher than the normal, healthy group. This small study, which lacked appropriate control groups for comparison, led the Gulf War Health Center to contact Fishbein for a more rigorous study. "We're now preparing to do a full study of 100 patients, with masked samples and matched controls," he notes.

There will be 25 patients in each of four groups: Gulf War veterans with symptoms; Gulf War veterans without symptoms; asymptomatic recruits who were not in the Gulf War; and military personnel with immunologic disease who were not in the Gulf War. Fishbein's group will utilize the nested PCR assay to obtain highly sensitive results for all 100 cases, then forward their findings back to the Gulf War Health Center to break the code for a final statistical comparison. "If there is a significant difference in the chromosomal changes in this study," he says, "it will suggest a possible exposure during the Gulf War. It won't tell us what it is, but it will force us to focus on potential environmental agents."

In addition to these two projects, multiple other PGI investigations are ongoing at the AFIP in the aforementioned departments and the Department of Veterinary Pathology.



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2nd Abdominal & Pelvic Imaging Review	12-13 April 97	Washington Marriott, Washington, DC
Interdisciplinary Course on Breast Disease	25-26 April 97	Leavey Conference Center, Georgetown University, Washington, DC
Lymph Node Interpretation: A Glass Slide Workshop	26 April 97	AFIP, Washington, DC
7th Anatomic Pathology Update & Review	11-17 May 97	Leavey Conference Center, Georgetown University, Washington, DC
3rd Musculoskeletal Imaging Weekend	17-18 May 97	Loew's Hotel, Annapolis, MD
Interpretation of Prostatic Biopsy	24-25 May 97	TBA, Silver Spring, MD
10th Annual Forensic Anthropology	9-13 June 97	USUHS, Bethesda, MD
6th Annual Descriptive Veterinary Pathology	10-13 June 97	AFIP, Washington, DC
12th Annual Diagnostic Exfoliative & Fine Needle Aspiration Cytology	16-20 June 97	AFIP Washington, DC
Telepathology Workshop	1-3 July 97	USUHS, Bethesda, MD
Descriptive Veterinary Pathology	7-11 July 97	TBA, Liverpool, England
Neuropathology Long Course	8 July-26 Sept 97	AFIP, Washington, DC
Genitourinary Radiology	14-18 July 97	AFIP, Washington, DC
Congenital DNA Repair Deficiency in Humans	20-21 July 97	TBA, Poughkeepsie, NY
Musculoskeletal Radiology	21-25 July 97	AFIP, Washington, DC
Neuroradiology	4-8 August 97	AFIP, Washington, DC
44th Annual Pathology of Laboratory Animals	11-14 August 97	Natcher Center, NIH, Bethesda, MD
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ABSTRACTS OF RECENT PUBLICATIONS BY AFIP STAFF

Hodgkin's disease and an extranodal marginal zone B-cell lymphoma in the small intestine: an unusual composite lymphoma

Nadine S. Aguilera, M.D., Leonard N. Howard, M.D., Mark D. Brissette, M.D., Susan L. Abbondanzo, M.D.

We describe a 74-year-old man who presented with multifocal small bowel lesions, a large mesenteric mass, and enlarged mesenteric lymph nodes. In each of the extranodal sites and in two of three regional lymph nodes, there were classic histologic features of marginal zone B-cell lymphoma with adjacent areas of Hodgkin's disease, mixed cellularity subtype. Immunophenotypic analysis in the areas of low-grade B-cell lymphoma of mucosa-associated lymphoid tissue showed immunoreactivity for CD45RB and CD20 in the malignant small cell population. Conversely, the areas of Hodgkin's disease demonstrated positive immunoreactivity for CD15 and CD30 in the Reed-Sternberg cells and variants. Latent membrane protein for Epstein-Barr virus was also positive in the Reed-Sternberg cells and variants.

Mod Pathol. 1996;9:1020-1026

Unusual Tumors of the Appendix and Pseudomyxoma Peritonei

Norman J. Carr, MRCPATH, and Leslie H. Sobin, MD

This report covers hyperplastic and neoplastic lesions of the human vermiform appendix that, although unusual, are likely to be encountered by general surgical pathologists. Topics include epithelial neoplasms, hyperplastic polyps, diffuse epithelial hyperplasia, pseudomyxoma peritonei, goblet cell and tubular carcinoid tumors, neuroma, and neurogenous hyperplasia. We discuss recent advances in the prognosis and management of appendiceal carcinoma, current controversies regarding the relationship between ovarian and appendiceal epithelial neoplasms, the concept of the mucinous tumor of uncertain malignant potential (UMP), and practical aspects of reporting pseudomyxoma peritonei. Classical carcinoid tumors are beyond the scope of this article, and primary neoplasms that are so rare they occur in the literature only as isolated case reports are not covered.

Semin Diagn Pathol. 1996;13:314-325.

Morbilliviral epizootic in bottlenose dolphins of the Gulf of Mexico

Thomas P. Lipscomb, Seamus Kennedy, Deborah Moffett, Amy Krafft, Brenda A. Klauenberg, Jack H. Lichy, Gerald T. Regan, Graham A. J. Worthy, Jeffery K. Taubenberger

Morbillivirus infection was diagnosed in 35/67 bottlenose dolphins (*Tursiops truncatus*) from the Gulf of Mexico that stranded from October 1993 through April 1994 in Alabama, Mississippi, and Texas (USA) during periods of increased dolphin strandings in each of the 3 states. Diagnosis was based on histologic lesions, immunohistochemical demonstration of morbilliviral antigen, and detection of morbilliviral RNA by a reverse transcriptase polymerase chain reaction (RT-PCR) test performed on formalin-fixed, paraffin-embedded tissue (5 dolphins), on histologic lesions and detection of morbilliviral RNA by RT-PCR performed on formalin-fixed, paraffin-embedded tissue (1 dolphin), and on detection of morbilliviral RNA by RT-PCR performed on unfixed lung samples collected from carcasses with advanced postmortem autolysis (29 dolphins). Histologic lesions included proliferative interstitial pneumonia with syncytial cells and eosinophilic intranuclear and intracytoplasmic inclusion bodies, lymphoid depletion and syncytial cells with eosinophilic intranuclear inclusion bodies in lymph nodes, eosinophilic intracytoplasmic inclusion bodies in transitional epithelium of urinary bladder, and a syncytial cell with eosinophilic intranuclear inclusion bodies in epidermis. Concomitant pulmonary aspergillosis was diagnosed histologically in 4 dolphins. This is the 5th reported morbilliviral epizootic of aquatic mammals and the 2nd involving bottlenose dolphins in the United States.

J Vet Diagn Invest. 1996;8:283-290.

Salivary gland cystadenocarcinomas: a clinico-pathologic study of 57 cases

R.D. Foss, CDR, DC, USN, G.L. Ellis, D.D.S., and P.L. Auclair, CAPT, DC, USN

Current classification schemes for salivary gland neoplasms categorize cystadenocarcinomas on the basis of a recurring histomorphologic pattern of cystic, and often, papillary growth without features of other specific types of salivary gland tumors. To ascertain the clinicomorphologic spectrum and biologic behavior of this tumor, the clinicopathologic features of 57 cystadenocarcinomas from the files of the Armed Forces Institute of Pathology were studied. Excluding five Veterans Administration military cases, men and women were equally affected. Patients ranged in age from 20 to 86 years (mean, 58.8; median, 64), and patients aged over 50 years accounted for 71% of cases. Thirty-seven tumors (65%) occurred in major salivary glands, 35 in the parotid, and two in the sublingual glands. The 20 minor salivary gland tumors (35%) involved, in descending order, the lips, buccal mucosa, palate, tongue, retromolar area, and floor of mouth. Grossly, the lesions were cystic or multicystic masses that ranged in size from 0.4 to 6.0 cm. Microscopically, all tumors demonstrated an invasive, cystic growth pattern, and 75% had a conspicuous papillary component. The predominant cell type varied among tumors and included small cuboidal cells (35 cases), large cuboidal cells (nine cases), and tall columnar cells (seven cases). Six cases exhibited an admixture of cell types. Ruptured cysts with hemorrhage and granulation tissue were common. All 40 patients with follow-up data were either alive or had died of other causes and were free of tumor a mean interval of 59 months after their initial surgery. Three tumors recurred locally (mean interval, 76 months). Three tumors were metastatic to regional lymph nodes at the time of diagnosis, and one patient developed a regional lymph node metastasis after 55 months. Salivary gland cystadenocarcinomas represent a distinct group of malignancies that have an indolent biologic behavior.

Am J Surg Pathol. 1996;20:1440-1447.

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2. Albores-Saavedra J, Gersell D, Gilks CB, Henson DE, Lindberg G, Santiago H, Scully RE, Silva E, Sobin LH, Tavassoli F, Travis WD, Woodruff JM. Terminology of endocrine tumors of the uterine cervix: results of a workshop sponsored by the College of American Pathologists and the National Cancer Institute. *Arch Pathol Lab Med.* 1997;121:34-39.
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6. Litovsky SH, Burke AP, Virmani R. Giant cell myocarditis: an entity distinct from sarcoidosis characterized by multiphasic myocyte destruction by cytotoxic T cells and histiocytic giant cells. *Mod Pathol.* 1996;9:1126-1134.
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9. Przygodzki RM, Finkelstein SD, Lange JC, Swalsky PA, Fishback N, Bakker A, Guinee DG, Koss M, Travis WD. Analysis of p53, K-ras-2, and C-raf-1 in pulmonary neuroendocrine tumors: correlation with histological subtype and clinical outcome. *Am J Pathol.* 1996;148:1531-1541.
10. Suster S, Moran CA. Primary thymic epithelial neoplasms showing combined features of thymoma and thymic carcinoma: a clinicopathologic study of 22 cases. *Am J Surg Pathol.* 1996;20:1469-1480.